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Viability of Embryos of the Winter Flounder 'Pseudopleuronectes americanus' Exposed to Combinations of Cadmiun and Salinity at Selected Temperatures

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Viability of Embryos of the Winter Flounder *Pseudopleuronectes americanus* Exposed to Combinations of Cadmium and Salinity at Selected Temperatures

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Abstract

Developing eggs of the winter flounder *Pseudopleuronectes americanus* (Walbaum) were exposed to 9 combinations of cadmium and salinity at 5° and 10°C. Overall mean times to 50% hatch ranged from 7.7 days at 10°C to 17.9 days at 5°C. Mean percentages of total hatches ranged from 50 to 100% for all treatment combinations. Response-surface analyses indicate percentages of viable hatches were generally lowest at 10‰ S and highest in the 25 to 30‰ S range. Regression analyses of viable hatch data show that at both temperatures cadmium significantly influenced viable hatch in all experiments. Viable hatch was also significantly influenced by the linear and quadratic effect of salinity in both tests at 5°C and in 2 of 3 tests at 10°C. The interaction between cadmium and salinity also significantly affected viable hatch at 10°C.

Introduction

Our goal was to evaluate effects of combinations of cadmium and salinity on the viability of embryos of the winter flounder *Pseudopleuronectes americanus* (Walbaum) at selected temperatures. This fish is found along the Atlantic coast of North America from Labrador to Georgia (McCracken, 1963) and constitutes an important local commercial fishery throughout its range (Bigelow and Schroeder, 1953). For example, the USA total catch in 1973 amounted to 9.7 metric tons (F.A.O., 1973).

Winter flounder spawn from mid-winter to early spring in offshore waters and in estuaries (Bigelow and Schroeder, 1953). In the latter areas, eggs and developing embryos may be subjected to fluctuations in such parameters as temperature and salinity as well as simultaneous exposure to various pollutants. Cadmium, a heavy metal, may be found in the effluents of electroplating, textile printing, and chemical industries (McKee and Wolf, 1963) and is thus one such potential pollutant.

Results of earlier studies involving a multivariate approach to bioassay indicate that in some instances the responses of marine animals subjected to cadmium are influenced by the thermal and saline conditions of exposure. For example, mummichogs, *Fundulus heteroclitus*, were more sensitive to this metal at 20° than at 5°C (Eisler, 1971); fiddler crabs, *Uca pugilator*, were least resistant when exposed to cadmium in combination with high temperatures and low salinities, 30°C-10‰ (O'Hara, 1973); survival and percentage viable hatch of herring eggs, *Clupea harengus*, exposed to the metal decreased at salinities of 16‰ and lower (von Westernhagen *et al.*, 1974). In comparison, survival and viable hatch of Baltic flounder, *Pleuronectes flesus*, embryos exposed to cadmium were unaffected by salinities of incubating waters between 16 and 42‰ S (von Westernhagen and Dethlefsen, 1975).

Materials and Methods

A randomized design involving a 3^2 factorial array of treatments was used in conducting experiments. In all instances each of the 9 treatments was repeated three times with 40 eggs of *Pseudopleuronectes americanus* (Walbaum) exposed per replicate observation. In addition, 1 control treatment at each salinity was added to the basic design in all experi-

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ments. Two sets of tests were conducted, with 3 experiments at 10°C and 2 at 5°C. Salinities of 10, 20, and 30‰ were tested at both temperatures. Nominal Cd^{+2} concentrations were 0.1, 0.32, and 1.0 mg l^{-1} at 10°C, and 1.15, 1.55, and 2.1 mg l^{-1} at 5°C.

Seawater used in preparing test dilutions was taken from Narragansett Bay in Rhode Island, USA, and typically had a salinity of about 30‰. Before use the water was passed through a multi-media filter and, where necessary, diluted to 10 or 20‰ S with deionized tapwater. Two hundred ml of dilution water at either 10, 20, or 30‰ S were added to each of the 30 assay containers, 100 ml polypropylene cups with bottoms of Nitex[1] screening inserted into 250 ml Pyrex beakers. One ml aliquots of appropriate stock solutions of $CdCl_2 \cdot 2\ ½\ H_2O$ were then added to each container. Containers of test media were loosely covered with either the tops or bottoms of Petri dishes in order to minimize evaporation of test media. Final cadmium levels were measured using an atomic absorption spectrophotometer and routine analytical techniques.

Mature *Pseudopleuronectes americanus* were taken by trawl net from Narragansett Bay and held 18 to 60 days in the laboratory in flowing seawater at ambient salinity (~30‰) and temperatures (~1° to 6°C). For each experiment, eggs stripped from a single female were fertilized with the pooled milt from 2 to 3 males. Fertilized eggs were then handled using procedures outlined by Smigielski and Arnold (1972). Aliquots of fertilized eggs were incubated for 24 h at either 5° or 10°C in filtered and aerated seawater at 30‰ S. Following incubation and immediately after the addition of cadmium to containers, groups of 40 eggs were counted under a dissecting microscope and transferred to assay containers using a Pasteur pipette. At the time of transfer, eggs incubated at 10°C were in early to mid-blastula stage. Those at 5°C were generally at late cleavage, with a few entering early blastula. During incubation and treatment eggs were exposed to continuous fluorescent lighting having an intensity of about 40 ft-c.

Assays were checked daily, and the number of larvae having hatched were counted and removed from test containers. Larvae which were completely free of chorions, morphologically normal in appearance, and able to swim were considered to be viable. All others were counted as dead. Tests were continued until completion of hatching or until it became apparent that no further hatch would occur.

Response-surface methodology was used to describe the relationship between percentage viable hatch and the cadmium-salinity treatments (Myers, 1971). Prior to analysis, an arc-sine transformation tabulated by Mosteller and Youtz (1961) was applied to the total percentage viable hatch data in each replicate observation, in order to stabilize the variance. Surfaces were approximated using the basic linear model

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_1X_1^2,$$

where Y = % response in angular units; X_1 = salinity; X_2 = log cadmium concentration. Results of each experiment were analyzed several times using variations of the basic model in which selected terms of the equation were deleted. The predictive model having the smallest mean square error was used in approximating response surfaces. Incubation times to 50% hatch (T_{50}) were interpolated using a probit transformation of the cumulative total hatches recorded in each 24 h observation interval and a logarithmic time scale. Rates of development (%/day = 100 T_{50}) were calculated from mean times to 50% hatch (Alderdice and Forrester, 1968).

Results

Total hatches of *Pseudopleuronectes americanus* (Table 1) in control treatments generally exceeded 85%, except in Test no. 2 at 10°C and 20‰ S, where only 55% of the eggs hatched. Reduced hatch in that one instance is most probably only an artifact reflecting the clumping of eggs which occurred within the assay container. Mean total hatches at 10°C were lowest (means 50 to 89%) among eggs in the high cadmium-low salinity regime, and consistently averaged at least 80% in each of the other treatments. At each level of salinity at 5°C, mean total hatches ranged from 90 to 100% at low and mid-concentrations of cadmium and from 60 to 88% at the high level of cadmium.

Lower percentages of viable larvae developed from cadmium-exposed eggs than from those incubated in control treatments (Table 2). Also, larval viability was generally less among cadmium-treated eggs at 10‰ S than at 20 and 30‰ S, a trend more pronounced at 5° than at 10°C.

Analyses of percentage viable hatch data (Table 3) show that, at 10°C, re-

[1] Mention of specific products herein does not constitute endorsement by the U.S. Environmental Protection Agency.

Table 1. *Pseudopleuronectes americanus*. Percent total hatch of winter flounder eggs (n = 40 eggs/observation) in each experiment at various combinations of cadmium and salinity at 10° and 5°C. Mean concentrations of cadmium (mg l^{-1} ± 1 standard deviation) measured in assay containers are in parentheses. In each experiment, fertilized eggs from separate spawnings were exposed

Experimental temperature and test no.	10‰ S				20‰ S				30‰ S			
10°C												
1	(0	0.10 ±.01	0.32 ±.02	0.97 ±.02)	(0	0.09 ±.02	0.33 ±.01	1.01 ±.03)	(0	0.11 ±.01	0.32 ±.05	1.04 ±.03)
	89	90	78	60	93	90	88	93	100	100	83	93
		65	80	68		80	93	80		95	88	100
		88	80	38		70	80	98		100	85	98
		$\bar{x}$ 81	79	55		80	87	90		98	85	97
2	(0	0.07 ±.02	0.35 ±.08	1.06 ±.08)	(0	0.08 ±.02	0.29 ±.01	0.99 ±.04)	(0	0.08 ±.01	0.31 ±.03	1.02 ±.07)
	88	95	98	90	55	100	90	93	95	88	80	78
		95	98	93		85	93	83		83	85	83
		98	95	83		88	100	95		85	80	93
		$\bar{x}$ 96	97	89		91	94	90		85	82	85
3	(0	0.09 ±.02	0.35 ±.01	1.08 ±.01)	(0	0.07 ±.01	0.34 ±.06	1.14 ±.02)	(0	0.11 ±.01	0.39 ±.01	1.14 ±.01)
	100	100	98	55	95	98	98	93	98	90	88	95
		95	95	45		100	98	88		98	85	93
		93	90	50		95	90	80		98	93	90
		$\bar{x}$ 96	94	50		98	95	87		95	89	93
5°C												
4	(0	1.06 ±.08	1.61 ±.06	2.16 ±.02)	(0	1.29 ±.27	1.58 ±.02	1.91 ±.27)	(0	1.14 ±.04	1.62 ±.02	2.19 ±.03)
	98	98	90	78	100	100	98	100	100	100	98	48
		100	90	68		100	95	83		95	95	68
		98	90	78		100	100	80		100	93	73
		$\bar{x}$ 99	90	75		100	98	88		98	95	63
5	(0	1.30 ±.02	1.88 ±.05	2.56 ±.04)	(0	1.30 ±.01	1.91 ±.05	2.58 ±.05)	(0	1.26 ±.01	1.85 ±.03	2.12 ±.06)
	100	90	90	75	100	100	90	68	100	95	100	75
		100	98	48		100	100	58		95	95	75
		85	100	70		100	98	53		100	100	78
		$\bar{x}$ 92	96	64		100	96	60		97	98	76

sults of Tests 2 and 3 are best represented by the complete basic model and those of Test 1 by that variant form of the basic model having no quadratic term. Deletion of the interaction term from the basic model provides a best fit of both sets of results at 5°C. Analyses of these data also indicate that regression explains a significant portion of the total variance in each experiment, with cadmium being a significant component of that regression in all instances (Table 4). Percentage viable hatch was significantly influenced by linear and quadratic effects of salinity in both experiments at 5°C. At the high temperature, linear (Tests 1, 2) and quadratic (Tests 2, 3) effects of salinity similarly influenced viable hatch as did the interaction between cadmium and salinity (Tests 2, 3).

Response surfaces, based on regression equations in Table 3, representing the percentages viable hatches at 10°C, indicate differences in the patterns of response of eggs from the three spawnings (Fig. 1A, B, C). In Test 1 (Surface A), for any single concentration of cadmium, the percentage of viable larvae rose as the salinity of the test media was increased. In comparison, at the same temperatures, larval viability in Tests 2 and 3 (Surfaces B, C) in relation to cad-

Table 2. *Pseudopleuronectes americanus*. Percent viable hatch of winter flounder eggs (n = 40 eggs/observation) in each experiment at various combinations of cadmium and salinity at 10° and 5°C. Mean concentrations of cadmium (mg l^{-1} ± standard deviation) measured in 3 replicate assay containers are in parentheses. In each experiment, fertilized eggs from separate spawnings were exposed

Experimental temperature and test no.	10‰ S				20‰ S				30‰ S			
10°C												
1	(0	0.10 ±.01	0.32 ±.02	0.97 ±.02)	(0	0.09 ±.02	0.33 ±.01	1.01 ±.03)	(0	0.11 ±.01	0.32 ±.05	1.04 ±.03)
	89	90	60	0	90	90	85	35	95	98	78	40
		63	48	3		70	80	10		90	83	38
		88	68	0		63	78	13		100	78	40
		$\bar{x}$ 80	59	1		74	81	19		96	80	39
2	(0	0.07 ±.02	0.35 ±.08	1.06 ±.08)	(0	0.08 ±.02	0.29 ±.01	0.99 ±.04)	(0	0.08 ±.01	0.31 ±.03	1.02 ±.07)
	85	95	45	0	55	90	80	83	88	65	75	45
		90	65	3		78	90	78		80	63	33
		93	68	10		83	93	90		60	75	58
		$\bar{x}$ 93	59	4		84	88	84		68	72	45
3	(0	0.09 ±.02	0.35 ±.01	1.08 ±.01)	(0	0.07 ±.01	0.34 ±.06	1.14 ±.02)	(0	0.11 ±.01	0.39 ±.01	1.14 ±.01)
	100	95	53	5	95	98	93	33	98	90	85	60
		95	60	3		100	98	68		98	85	75
		93	65	5		95	90	50		95	90	65
		$\bar{x}$ 94	59	4		98	94	50		94	87	67
5°C												
4	(0	1.06 ±.08	1.61 ±.06	2.16 ±.02)	(0	1.29 ±.27	1.58 ±.02	1.91 ±.27)	(0	1.14 ±.04	1.62 ±.02	2.19 ±.03)
	98	50	15	13	95	78	85	58	95	88	90	35
		25	20	5		90	78	30		73	88	50
		28	15	0		85	70	60		83	75	35
		$\bar{x}$ 34	17	4		84	78	49		81	84	40
5	(0	1.30 ±.02	1.88 ±.05	2.56 ±.04)	(0	1.30 ±.01	1.91 ±.05	2.58 ±.05)	(0	1.26 ±.01	1.85 ±.03	2.12 ±.06)
	100	43	30	13	100	95	63	35	100	85	80	28
		53	30	0		93	78	23		90	80	43
		48	20	3		93	83	20		90	90	39
		$\bar{x}$ 48	27	5		94	75	26		88	83	36

Table 3. *Pseudopleuronectes americanus*. Multiple-regression equations for percentage viable hatch of eggs. Y = % response in angular units; X_1 = salinity, X_2 = log cadmium concentration

Experimental temperature and test no.	Equation
10°C	
1	$Y = -0.0157 + 0.0256X_1 - 1.0753X_2 + 0.0161X_1X_2$
2	$Y = -0.8934 + 0.1575X_1 - 1.3003X_2 + 0.0438X_1X_2 - 0.0033X_1^2$
3	$Y = -0.6170 + 0.1085X_1 - 1.4005X_2 + 0.0359X_1X_2 - 0.0018X_1^2$
5°C	
4	$Y = -0.4336 + 0.1451X_1 - 1.5270X_2 - 0.0029X_1^2$
5	$Y = 0.1131 + 0.1051X_1 - 2.3810X_2 - 0.0020X_1^2$
4 + 5	$Y = -0.1618 + 0.1251X_1 - 1.954X_2 - 0.0025X_1^2$

Table 4. *Pseudopleuronectes americanus*. Regression analyses for viable hatch of eggs exposed to 9 combinations of cadmium and salinity at 10° and 5°C. Arcsine transformation applied to percent responses in each experiment prior to analysis. DF: degrees of freedom; MS: mean square; F: ratio treatment mean square to error mean square; R^2: correlation coefficient

Analysis	Test 1 (10°C)				Test 2 (10°C)				Test 3 (10°C)				Test 4 (5°C)				Test 5 (5°C)			
	DF	MS	F	R^2	DF	MS	F	R^2	DF	MS	F	R^2	DF	MS	F	R^2	DF	MS	F	R^2
Regression																				
Log cadmium	1	2.553	82.3**	0.82	1	0.809	26.9**	0.76	1	2.098	174.8**	0.93	1	0.718	44.8**	0.87	1	1.745	134.2**	0.91
Salinity	1	0.558	18.0**		1	0.048	1.6		1	0.540	41.5**		1	1.389	86.8**		1	1.087	83.6**	
Interaction	1	0.078	2.5		1	0.576	19.2**		1	0.386	32.1**									
Salinity^2					1	0.639	21.3**		1	0.201	16.7**		1	0.517	32.3**		1	0.243	18.6**	
Error	23	0.031			22	0.030			22	0.012			23	0.016			23	0.013		
Corrected total	26	0.150			26	0.105			26	0.134			26	0.115			26	0.130		

**$F_{0.99}$ (1,23) = 7.88; $F_{0.99}$ (1,22) = 7.94.

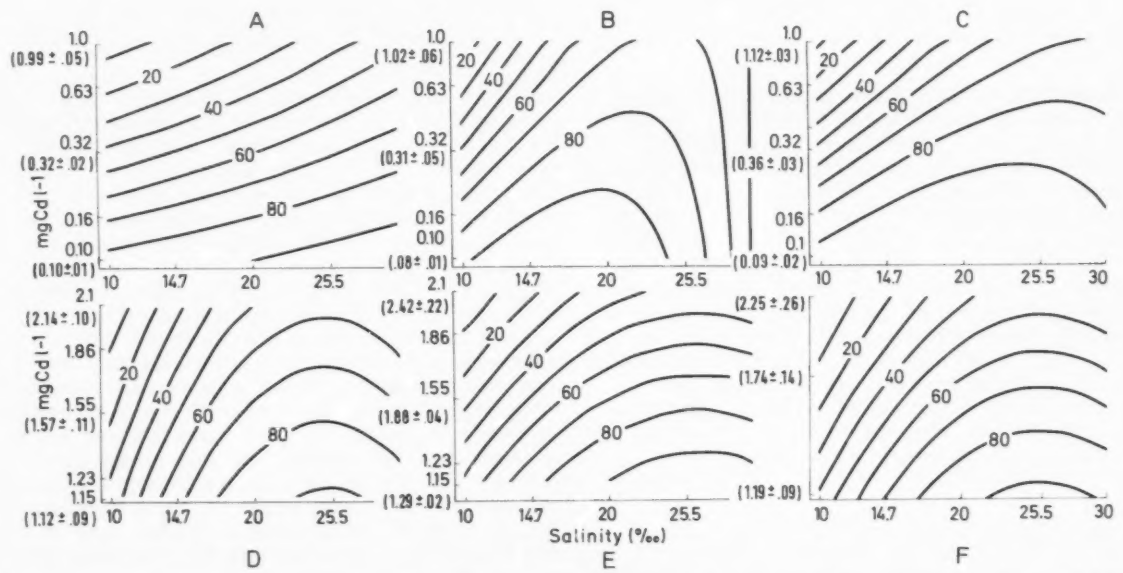

Fig. 1. *Pseudopleuronectes americanus*. Percentage viability of embryos exposed to combinations of cadmium and salinity. Measured concentrations of cadmium are in parentheses, other values for cadmium represent nominal levels of the metal. Salinities tested include 10, 20, and 30‰. Tests conducted at 10°C (Surfaces A, B, C) and 5°C (Surfaces D, E, and F). Each surface represents responses of eggs from separate spawnings

mium concentration was greater in approximately the 20 to 25‰ S range than at either higher or lower salinities. Cadmium-salinity interactions (Tests 2, 3) indicated by the regression analyses are also evident in Surfaces B and C, in terms of the rotation of the isograms relative to the main axes. Viability of larvae from eggs incubated at 5°C was also greater at about 25‰ S (Fig. 1D, E) than at either higher or lower salinities. Results of Tests 4 and 5 are not significantly different statistically, and pooling these results yielded Surface F in Fig. 1.

The greater consistency evident in the patterns of surface configurations representing low temperature data show a higher degree of uniformity in responses of eggs incubated at 5° than at 10°C. This consistency is also suggested by the comparatively lower and more uniform mean-square error terms and related correlation coefficients (R^2), indicating regression equations provided better fits for 5° than for 10°C data (Table 4).

Table 5. *Pseudopleuronectes americanus*. Days from fertilization to 50% hatch (T_{50}) and rates (%/day = $\frac{100}{T_{50}}$) of development of eggs in varying cadmium-salinity-temperature conditions. T_{50} values are means of the 3 observations of each treatment ± 1 standard deviation. For each temperature, concentrations of cadmium (mg l^{-1}) shown in parentheses are the overall mean values of measurements in all experiments at each level of the metal

Experimental temperature and test no.		10‰ S				20‰ S				30‰ S			
10°C		(0.0	0.09	0.34	1.04)	(0.0	0.08	0.32	1.05)	(0.0	0.10	0.34	1.06)
1	T_{50}	7.67	7.71±.20	8.45±.34	9.02±.81	7.59	7.97±.30	7.84±.09	7.52±.31	7.67	7.71±.36	7.59±.26	7.03±.52
	%/day	13.04	12.97	11.83	11.09	13.18	12.55	12.76	13.30	13.04	12.97	13.18	14.23
2	T_{50}	7.85	7.85±.09	7.66±.08	7.57±.21	7.00	8.01±.26	8.27±.52	7.66±.01	7.94	7.46±.16	7.96±.86	7.18±.36
	%/day	12.74	12.74	13.06	13.21	14.29	12.48	12.09	13.06	12.59	13.41	12.56	13.93
3	T_{50}	7.16	7.67±.23	7.34±.16	8.5 ±.70	7.24	7.72±.11	7.76±.09	7.59±.16	7.08	7.80±.12	7.69±.24	7.34±.14
	%/day	13.97	13.04	13.62	11.77	13.81	12.95	12.89	13.18	14.12	12.82	13.00	13.62
5°C		(0.0	1.18	1.75	2.36)	(0.0	1.30	1.75	2.25)	(0.0	1.20	1.74	2.16)
4	T_{50}	18.45	17.9±.25	18.3±.28	17.2±.17	16.98	17.4±.10	17.4±.11	17.4±.64	17.91	17.1±.40	17.4±.05	17.1±.70
	%/day	5.42	5.59	5.47	5.81	5.89	5.75	5.75	5.75	5.58	5.85	5.74	5.85
5	T_{50}	20.75	18.5±.17	18.9±.29	19.2±.35	19.54	18.2±.25	18.1±.20	19.3±.65	19.32	18.1±.26	17.7±.28	18.3±.47
	%/day	4.82	5.41	5.29	5.21	5.12	5.50	5.53	5.18	5.18	5.53	5.65	5.47

Standard deviations associated with the times to 50% hatch (Table 5) suggest that rates of development were highly variable, with no apparent correlation between patterns of response and individual treatments. However, in terms of overall means, a comparison of developmental rates for unexposed and cadmium-exposed eggs indicates that those eggs exposed to cadmium tended to develop somewhat more slowly than controls at 10°C, and slightly more quickly at 5°C. A similar comparison of overall means with salinity indicates a more rapid development at the high salinity than at the low or mid-level salinity.

Discussion

A comparison of percentage responses for total and viable hatches in *Pseudopleuronectes americanus* shows that larvae from cadmium-exposed embryos were generally less viable than were those from control treatments. These differences suggest that, although embryonic survival was high in all treatments, cadmium had a deleterious impact on embryological development that manifested itself at the time of hatching. This interpretation is supported by both the unpublished results of an exploratory experiment we conducted, which showed that newly hatched larvae exposed to cadmium are able to survive concentrations of at least 1.0 mg l^{-1} for 4 days at 10°C and 10‰ S, and by the graded effect of cadmium on viable hatch in these tests. The more sensitive nature of viable hatch, as compared to the embryonic survival implied by total hatch data, is particularly evident at 10‰ S. At 10‰ S, many larvae that developed from embryos incubated at intermediate and high levels of cadmium were malformed, and increased mortality at the time of hatching was evident. Similar incidents of malformations and increased mortality have been associated with incubation of embryos of other fishes at low salinity (Battle, 1930; Holliday, 1965). The patterns of response observed in this study and the similar observations of others suggest that the exposure to cadmium augmented any effect low salinity may have had on development. This effect may be related to a greater uptake of water by the developing eggs at the low salinity, as has been reported to occur in the eggs of a number of marine fishes prior to completion of gastrulation (Holliday, 1965), and a concomitant increase in the uptake of cadmium.

Concentrations of cadmium estimated to yield 50% (EC_{50}) and 75% (EC_{75}) viable hatches serve to illustrate the general nature of the responses depicted in Fig. 1. At 10°C, for example, EC_{50}'s ranged upward from about 0.3 mg Cd l^{-1} in all tests at 10‰ S to about 1.0 mg l^{-1} at 30‰ S in the first test, and to beyond the range of concentrations of the metal tested at 20 and 30‰ S in the second and third experiments. EC_{75} concentrations at 10°C were also least at 10‰ S (about 0.16 mg l^{-1}) and approximately 0.8 mg l^{-1} at 30‰ S in Test 3. Similar patterns of response were also evident at 5°C, with greatest sensitivity at 10‰ S (EC_{50}'s of 1.1 to 1.3 mg l^{-1}) and maximum resistance in the 20 to 30‰ S range (EC_{50}'s and EC_{75}'s of ~2.0 mg l^{-1})

Our interpolated EC_{50} and EC_{75} values are substantially higher than the 0.06 and 0.11 µg Cd l^{-1} reported in continen-

tal shelf waters of the United States (Windom and Smith, 1972). They are, however, comparable to the mean concentrations of 1.4 (Windom, 1975) and 4.0 mg Cd l^{-1} (Harding and Brown, 1975) measured in sediments taken from estuaries receiving cadmium-containing wastes. It is thus possible that the demersal egg of the winter flounder, through contact with sediments, could be exposed to levels of cadmium shown to reduce embryo viability. The potential toxic effects of cadmium-bound sediments are, however, not yet clear.

Comparison of our results with those reported for herring (von Westernhagen *et al.*, 1974) and Baltic flounder (von Westernhagen and Dethlefsen, 1975) indicate that, in terms of viable hatch, resistances of these species to cadmium are similar. Further comparisons show that both winter flounder and herring were more sensitive to the toxic effects of cadmium at low salinities, but that the viability of Baltic flounder larvae was unaffected by salinity during incubation. However, surface analyses of our data suggest that, unlike the resistance of herring, that of winter flounder to cadmium tended to decrease with increases in salinity above 25‰.

Our estimates of cadmium effects on viable hatch were based on total amounts of the metal measured in assay containers. The model provided by Zirino and Yamamoto (1972) indicate that the most prevalent species of the metal were $CdCl_2^0$ (51%), $CdCl^+$ (39%), $CdCl_3^-$ (6%), and Cd^{+2} (2.5%). Except in one case, measured concentrations closely approximated nominal levels of cadmium added to assay containers. The difference in that one instance stems from a technical error in the handling of stock solutions.

The greater viability we observed at 20 to 25‰ S is evident at both experimental temperatures, and is consistent with the findings noted by Rogers (1976), who suggested the 15 to 25‰ salinity range as being optimal for the embryological development of winter flounder at temperatures above 3°C. The relative inconsistencies in responses at the high temperature in our study correspond to both the greater variability at 12°C and the lower viability at temperatures above 10°C described by Williams (1975) from his study of temperature effects on embryos of winter flounder. Williams also recorded a reduction in Q_{10} associated with rate of embryonic development at temperatures above 12°C, and suggested that the decrease might be indicative of stress. Development of cadmium-salinity interaction in our tests at 10°C similarly might also be interpreted as reflective of incipient stressful conditions. Conversely, in tests at 5°C (a temperature which approaches the optimum for embryogenesis of 3°C reported by Rogers, 1976), responses are more consistent and show no evidence of interdependence between cadmium and salinity.

Overall mean estimates of times to 50% hatches range from 7.7 to 17.9 days at 10° and 5°C, respectively and are substantially the same as those reported by Williams (1975). They are, however, somewhat faster than rates of development recorded by Rogers (1976). The slightly shorter incubation periods at higher salinities that we observed do tend to corroborate the developmental rate-salinity relationships reported by Rogers. Comparison of our estimates by temperature show a more pronounced effect of salinity at the low than at the high experimental temperature, a difference which may only reflect the higher degree of variability in test results at 10° than at 5°C. Faster rates of development at higher salinities have also been noted in studies of embryos of other fishes including English sole, *Parophrys vetulus* (Alderdice and Forrester, 1968) and Pacific cod, *Gadus macrocephalus* (Alderdice and Forrester, 1971).

Effects of toxicants on the aquatic life of estuaries could perhaps be better evaluated through the more routine use of the type of experimental design and data analysis we used here. This response-surface approach would provide regulatory agencies with an alternative methodology of value in screening the toxicities of potential pollutants over a range of experimental conditions. Information generated in this manner would thus provide insight into the effects of interactions which may exist between a toxicant and an environmental factor. This method will yield an overall pattern of response that is more generally applicable to the formulation of water quality criteria than the finite values representing medium tolerance concentrations presently used.

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